Methods for Estimating Filamentous Algae Cover in Streams and Rivers of the Shenandoah River Basin

Report Second Draft

March 12, 2015

Adam N. Griggs
Gordon Michael Selckmann
James Cummins
Claire Buchanan

Prepared for

U.S. EPA Region 3
Office of Standards, Assessment and TMDLs
Water Protection Division
1650 Arch Street, Philadelphia, PA 19103

by

Interstate Commission on the Potomac River Basin
30 West Gude Dr, Suite 450
Rockville, MD 20850
301-984-1908
www.potomacriver.org

United States
Environmental Protection
Agency

ICPRB Report 15-1

To receive additional copies of this report, please write or call

Interstate Commission on the Potomac River Basin
30 West Gude Dr, Suite 450
Rockville, MD 20182
301-984-1908

After the report has been reviewed and released for distribution by the Environmental Protection Agency, an electronic copy of the report can be downloaded from the Publications tab at www.potomacriver.org.

Disclaimer

The opinions expressed in this report are those of the authors and should not be construed as representing the opinions or policies of the United States government, or the signatories or Commissioners to the Interstate Commission on the Potomac River Basin.

Acknowledgements

This project was supported by a grant from the Environmental Protection Agency (CWA§106 grant 98339412-0) and by the Interstate Commission on the Potomac River Basin, an interstate compact river basin commission of the United States government and the compact's signatories: Maryland, Virginia, Pennsylvania, West Virginia, and the District of Columbia.

The project would like to acknowledge the technical and policy support provided by the Environmental Protection Agency, the West Virginia Department of Environmental Protection, and the Virginia Department of Environmental Quality. In addition, we would like to thank the citizen monitors and their parent organizations, the Friends of the North Fork and the Friends of the Shenandoah River.

TABLE OF CONTENTS

LIST OF APPENDICES

ABBREVIATIONS

AFDM	Ash free dry mass
ANOVA	Analysis of variance
BMP	Best management practice
CWA	Clean Water Act
EPA3	United States Environmental Protection Agency, Region 3
EMAP	Environmental Monitoring and Assessment Program
FoSR	Friends of the South River
FoNFSR	Friends of the North Fork Shenandoah River
GPS	Global positioning system
ICPRB	Interstate Commission on the Potomac River Basin
NAWQA	North American Water Quality Assessment

NRSA	National River and Streams Assessment
TMDL	Total Maximum Daily Load
RBP	Rapid Bioassessment Protocol
RPS	Rapid Periphyton Survey
SOP	Standard operating procedure
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
VADEQ	Virginia Department of Environmental Quality
WVDEP	West Virginia Department of Environmental Protection

EXECUTIVE SUMMARY

This report presents the results of a pilot study to investigate and develop methods for measuring filamentous algae. The study area was the Virginia's Shenandoah River basin. The objectives were to 1) develop quantitative, repeatable, and scientifically valid methods for measuring filamentous algal growth in the Shenandoah River and its tributaries that can be applied in Virginia's other non-tidal (free-flowing) waters; and 2) investigate the feasibility of using citizen monitors to collect algal data in a manner acceptable to VADEQ and EPA.

Filamentous algae are single cells joined end-to-end to form dense hair-like mats or coarse entwined cords, sometimes meters-long, which can cover stream and river bottoms. Dense blooms of filamentous algae can harm aquatic ecosystems and interfere with recreational uses. Protocols for collecting, analyzing, and quantifying filamentous algae in streams and rivers are rare. West Virginia Department of Environmental Protection (WVDEP) has developed a method for visually estimating percent cover of filamentous algae across a lateral transect of a stream or wadeable river.

This pilot study adapted the WVDEP method to sites on non-wadeable rivers, and for longitudinal surveys. The study also developed a subsampling approach for longitudinal surveys that can facilitate areal estimates of algal growth. A two-person crew can rapidly implement the lateral transect methods at wadeable and non-wadeable sites. Longitudinal surveys are more time and effort intensive, and might be best implemented in river reaches with known or suspected algae blooms, during the peak algal growing season.

It is essential for field observers to be able to distinguish filamentous green algae (Chlorophyta) from from other forms of aquatic vegetation, including blue-green algae (Cyanophyta), vascular plants, and the crust-like periphyton, which are complex assemblages of bacteria, algae, and fungi. These algae and plant types often occur together, attached to the same substrates in streams and rivers. Robust training and, if possible, certification in identifying the major algae and plant taxonomic groups is important for any field observers undertaking river watches or surveys. With training, volunteer monitors could assist state biologists in finding algae-prone areas of river systems. Volunteer data may be considered with other evidence, including agency routine monitoring data, in locating and tracking areas of filamentous algae growth.

Comparisons of visual estimates of percent algal cover, made by trained observers in the field, indicate close agreement. This pilot study tested the potential of image processing software as a tool for independently verifying visual estimates. ImageJ software, developed by the National Institutes of Health, was applied to photos representing different levels of algal cover in the field and compared to visual estimates made from the original photos. Imaging software appears to have potential as an auxiliary protocol for quantifying algal cover, a validation tool for field data, and may be useful as a training tool.

The methods developed and tested for this pilot study can be adapted for a variety of monitoring and research needs. They can be rapidly employed when "nuisance plant growth" and filamentous algal blooms are reported.

1. ISSUE STATEMENT

Citizen complaints and news reports (e.g., *Bay Journal* 2013) of excessive filamentous algae in the streams and rivers of the Shenandoah River watershed prompted discussions in 2013 between staff from the U.S. Environmental Protection Agency Region III (EPA3) and Virginia Department of Environmental Quality (VADEQ). Both parties recognized the need for a scientifically valid method of quantifying filamentous algae that would allow VADEQ to evaluate algal growth in Virginia's non-tidal (free-flowing) waters, and they agreed to work cooperatively toward that end (**Appendix A**). Acknowledged in the agreement was the potential of citizen monitors to collect good quality data on filamentous algae distributions.

EPA3 provided support to the Interstate Commission on the Potomac River Basin (ICPRB) in 2014 to explore and test possible field methods in a pilot study on Virginia's Shenandoah River. The pilot study had two objectives: 1) develop quantitative, repeatable, and scientifically valid methods for measuring filamentous algal growth in the Shenandoah River and its tributaries that can be applied in Virginia's other non-tidal (free-flowing) waters; and 2) investigate the feasibility of using citizen monitors to collect algal data in a manner acceptable to VADEQ and EPA. This report describes the results of the pilot study.

2. BASIC ECOLOGY OF FILAMENTOUS GREEN ALGAE

Filamentous algae are aquatic colonies of cells joined end-to-end to form long, visible chains that have hair- or rope-like appearances. They are natural elements of biological communities in aquatic systems. Taxa that live in moving waters are usually attached to hard surfaces such as rocks, aquatic plants and woody debris. Filaments can fragment and become free-floating in the water column after scouring by high flow events, and will disperse downstream where they can recolonize (Wehr and Sheath 2002). Ecological research on filamentous algae has focused on lake, pond, and marine environments. Filamentous algae in streams and rivers, and especially large rivers which can be difficult to study, are less studied but beginning to receive more attention.

The visible, or macroscopic, taxa of filamentous algae are primarily Chlorophyta, also called green algae. Chlorophyta are a very large, diverse group of the "true" algae that can undergo both sexual and asexual reproduction, and whose cells have well-formed nuclei, chloroplasts, and other organelles. Commonly encountered types of filamentous green algae in rivers include coarse, entwined, multi-species colonies that form mats or cords several meters in length. In full bloom, filaments may entirely cover the bottom substrate, and extend through the water column to dominate the surface. Taxa producing this type of filamentous algae include *Spirogyra*, *Cladophora*, *Oedegonium*, *Rhizoclonium*, and *Chaetomorpha*.

Along with other benthic (attached) algae, filamentous green algae are considered one of the primary autotrophic sources of organic matter in rivers and large streams. They act as chemical modulators in aquatic systems, transforming inorganic chemicals into organic forms through photosynthesis (Lock et al. 1984), and can be important sinks for phosphorus and nitrogen (Wetzel, 2001). Filamentous green algae can be consumed by microorganisms, snails, insects, and fish (Vannote et al. 1980) and serve as refuge for invertebrates and fish (Copp 1997). The algae exhibit seasonal cycles, generally growing more rapidly as waters warm and sunlight increases. Some genera, such as *Spirogyra*, grow better in cooler temperatures and lower light conditions and tend to be more abundant in the spring and fall (Wehr and Sheath 2002).

Filamentous green algae often attain their greatest sizes in moving waters (Whitford 1969; Raven 1992). Due to the algae's capacity for rapid cell division, nutrient enrichment can quickly result in excessive growth, degrading ecosystem structure (Stevenson *et al*. 1996). Excessive algae may smother or displace benthic macroinvertebrates (Quinn and Hickey 1990) and may reduce dissolved oxygen and change pH, which can lead to fish kills (Quinn and Gilliland 1989). Large algal taxa like *Cladophora* and *Rhizoclonium* are capable of overwhelming vascular plant communities, and they negatively impact aesthetics (Welsh *et al*. 1988). In enriched slow-moving water these species usually form dense mats, but in flowing water they can become long, rope-like strands (Welsh *et al*. 1988). Very high densities of algae, including filamentous green algae, may impart distasteful flavors to drinking water as they die and decompose (Speer 2015).

While growth in lakes tends to be limited by water column concentrations of phosphate, a more important limiting factor in rivers can be light availability (Schindler 1977). Algae in forested streams or along river edges can exhibit their greatest growth in early spring or late fall when leafless trees permit light to reach the water (Klapproth and Johnson 2000). Hydraulic flushing, which scours and fragments filamentous algae, is another important factor controlling algal growth. Rivers with short-retention times, such as those with steep gradients, will manifest different algal responses to nutrient enrichment compared to rivers with low gradients and long retention-times. Long retention-time rivers will operate more like lakes, moving from macrophyte to phytoplankton domination with nutrient enrichment whereas short retention-time rivers tend to move from macrophyte to benthic and filamentous algae domination (Hilton et al, 2006).

3. Review of Existing Algal Sampling Methods

Several federal and state agencies have created algal bioassessment programs to quantify the abundance and characteristics of benthic algal communities, and have incorporated that information in biological indices (**Appendix B**). These efforts focus primarily on periphyton assemblages, and most often diatom taxa; they rarely focus on the long chain filamentous green algae. In this section, existing algal sampling protocols are reviewed for their potential use as a filamentous algae methodology.

The EPA describes periphyton sampling procedures in its *Rapid Bioassessment Protocols (RBP) for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition* (EPA 2012). Periphyton are complex assemblages of bacteria, blue-green algae, true algae (includes diatoms), fungi, and detritus that form rough, mat-like coatings on surfaces of submerged substrates. Periphyton sampling can produce both algal taxonomic and biomass metrics. Taxonomic diversity is investigated using a quantitative, targeted habitat approach or a qualitative, multiple habitat approach. The quantitative method relies on the identification of a "richest targeted habitat" (RTH), which is expected to support the richest taxonomic diversity in the river reach, and a "depositional targeted habitat" (DTH), which is the section of the river reach most exposed to sediment-borne contaminants for extended periods. The qualitative multihabitat sampling regime samples in every microhabitat where periphyton are likely to be present, in order to compile the most complete taxa list of the surveyed area. From these two methodologies, the EPA lists 15 different taxonomic metrics/indices that can be derived from the collected periphyton. The USGS uses similar algal protocols for its *National Water Quality Assessment Program (NAWQA): Algal Assessment Protocols for Non-wadeable Streams and Rivers* (Flotemersch, 2006). Its methods rely on the collection of two quantitative periphyton samples, one qualitative periphyton sample, and one phytoplankton sample. Multihabitat and single habitat methods are used to identify species richness and diversity in diatom and periphyton communities. Both the EPA and USGS protocols require taxonomic identification and enumeration of the sample contents, which can become cost prohibitive and, in cases with multiple taxonomists, lead to differences in species identification. These methods do not measure the spatial extent of algal cover in the stream or river and therefore are not well suited to measuring filamentous algal growth or abundance.

Laboratory protocols that produce estimates for whole community metrics from algal samples are particularly germane to the study of nutrient enrichment and toxicity in stream systems. These measures generally rely on chlorophyll *a*, ash-free dry mass (AFDM), total cell density, and measures of biovolume collected from a known substrate area. They are used to determine water quality impairment and explore relationships to environmental stressors. Inter-annual variability and the lack of reliable relationships with other variables have made biomass assessments less informative in some instances (SWAMP 2009). While less time consuming than the taxonomic enumerations, whole community estimates require a laboratory component that introduces potential inter-lab variability and additional processing costs. The whole community estimates are also sample-specific and do not address the spatial extent of algal cover in the stream or river.

Methodologies have been developed to approximate the spatial extent of algal cover from measurements made along transects in streams and rivers. Simple transect methodologies consist of algal presence/absence observations taken at defined points. Presence/absence methods alleviate the time and effort required to perform sample collections and taxonomic identification, and require little specialized training. One method that can be used independently or in conjunction with transect methods is the EPA Rapid Bioassessment Protocol (RBP) viewing bucket method. The viewing bucket is marked with a 50-point grid that is used for quick, semi-quantitative assessments of benthic algal biomass. This technique enables rapid assessment of algal biomass over moderately sized areas in the form of a mosaic made up of several samples. More rigorous, high density point transects with additional components such as specimen collections and taxonomic identification can be added. As is the case with the taxonomy-based methodologies, this protocol lacks the large-scale application needed to measure the spatial extent of filamentous algae in rivers.

The West Virginia Department of Environmental Protection (WVDEP) has developed a protocol specifically for quantifying the spatial extent (percent cover) of filamentous green algae in the state's streams and wadeable rivers. The protocol consists of a rapid bioassessment style survey with qualitative physical habitat evaluations, transect-segment algae percent cover estimates, and routine water quality and chemistry collection including: nitrate, nitrite, total Keldahl nitrogen, dissolved and total phosphorous, calcium, magnesium, and total alkalinity. The method is performed when filamentous algae appear in streams and rivers. The method's Standard Operating Protocol and monitoring field form can be found in **Appendices C** and **D**, respectively, and the method is discussed below in more detail. Both WVDEP and ICPRB staff have used the method in watersheds adjacent to the Shenandoah, specifically the Cacapon and South Branch of the Potomac River, and find it suitable for use throughout much of the upper and middle Shenandoah basin.

The EPA Environmental Monitoring and Assessment (EMAP) program developed an algal methodology to determine stream integrity of western USA streams (Lazorchakl et al. 1998). The program created a 1-5 narrative scale to describe a waterbody's condition. Water bodies were assigned a rating of 1 (highly disturbed) to 5 (pristine) based on the biologists' narrative interpretation of transects:

1. Enjoyment nearly impossible
2. Level of enjoyment substantially reduced
3. Enjoyment impaired
4. Very minor aesthetic problems; excellent for swimming, boating, enjoyment
5. Beautiful, could not be any nicer

The western EMAP narrative scale is able to quickly and qualitatively assess a river length as being impaired or at risk by algae, but is not able to provide numerical quantitative algal assessments.

With the exception of the WVDEP and EPA EMAP protocols, each of the methodologies described above are periphyton protocols aimed at collecting site-specific, taxonomic and/or biomass-related information. These data are incorporated in various ways into indices of stream biological health. The EPA EMAP pilot study and the WVDEP protocols are the only methods relating algal abundance to a recreational usage. Only WVDEP has developed numerical criteria for filamentous algae and used them to quantify impairment of "recreational use."

4. Study Area

The Shenandoah River is formed by the confluence of the North Fork Shenandoah and South Fork Shenandoah rivers in Front Royal, Virginia, herein referred to as the North Fork and South Fork or simply the Forks. These two main tributaries of the Shenandoah River are each about 100 miles in length and wind through fertile, limestone valleys to their confluence. From there, the Shenandoah River mainstem follows a generally northeast course for another 56 miles, traversing West Virginia's panhandle before it reaches the Potomac River at Harpers Ferry, West Virginia (**Figure 1**).

Rivers in the basin are relatively shallow with a hard cobble or bedrock bottom. Many reaches of the two Forks are less than 50 meters wide while the mainstem is as wide as 250 meters near its mouth. Portions of the Forks are shallow enough to wade across but other sections are too deep to wade. The North Fork and South Fork each make numerous bends and meanders. Although Routes 340 and 11 parallel the general courses of the South and North Forks, respectively, the rivers are not visible from the roads much of the time.

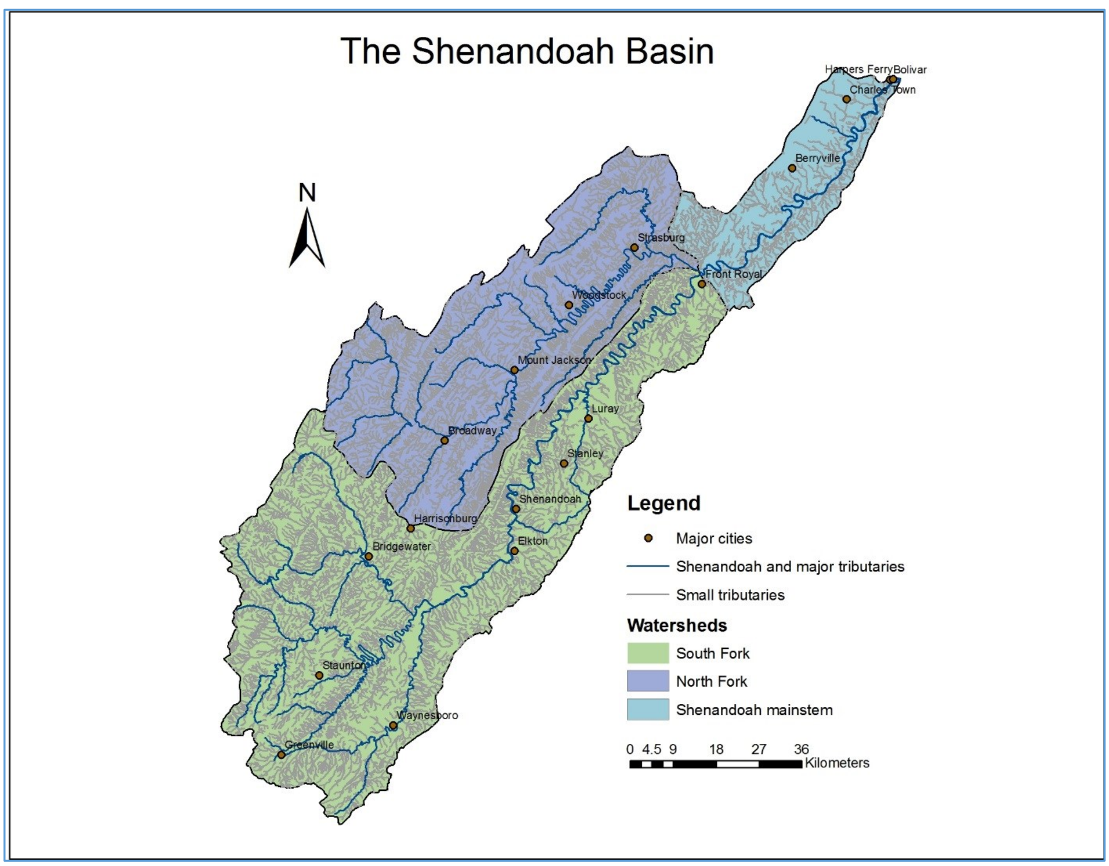

Figure 1 The Shenandoah River basin.

The Shenandoah River basin is a large watershed with a drainage area of 2,937 square miles. According to the 2006 National Land Cover Dataset, land cover in the Shenandoah watershed is 56% forest, 33% agriculture, 9.6% suburban and urban, and less than 1% other (water, wetlands, barren land, grasslands, shrub/scrub). Recreational uses of the Shenandoah River are popular, and include fishing, canoeing, river tubing, and white-water rafting and kayaking.

5. Sampling Design Considerations

This pilot project investigated several methods for measuring filamentous algae. Different methods were needed in order to contend with the range of river widths experienced in the Shenandoah River system, the inaccessibility of many reaches, and the fact that some sites are wadeable and others are not. The variability in filamentous algae abundance was also a factor influencing method design. Designing a filamentous algae monitoring program was outside the scope of this pilot study, but thought was given to how a selection of methods could make future monitoring programs adaptive and capable of addressing different monitoring objectives over time.

Method design issues that should be addressed before agency staff go into the field to find and measure filamentous algae are discussed below.

A. Reconnaissance

Knowledge of where filamentous algae are usually found in a river system can assist efforts to detect annual blooms, which are very high levels of algae. Filamentous algae establish themselves quickly, in patchy distributions, where conditions are favorable. They often recur in the same places, from year to year, if conditions remain favorable. Algae *blooms* are unpredictable in the sense that their timing and duration is variable, uncertain, and erratic. Recreational users of the river and state agency personnel collecting water quality and stream macroinvertebrate samples are most likely to observe bloom occurrences. Regular "wind-shield surveys" from nearby roads can also be an effective means of finding blooms if the roads allow for unobstructed views of the streams and rivers. The combined reports and observations from these various sources can provide the necessary information for identifying "hotspots," where algae blooms tend to form.

After bloom hotspots are identified, a flexible, community-watch approach during the algal growing season could prove helpful in tracking the onset, extent, and duration of the blooms. Capable volunteers are interested in their local areas, and members of long-running citizen monitoring programs have demonstrated their ability to collect water quality data on a regular basis. Their observations can support and enhance those made by trained, state agency biologists.

In the case of the Shenandoah, two citizen monitoring groups are actively engaged in collecting and analyzing water quality samples in Shenandoah waters: Friends of the South River (FoSR) and Friends of the North Fork Shenandoah River (FoNFSR). Their regular trips to monitoring sites give them opportunity to scan streams and rivers for blooms, and they have indicated willingness to monitor algal occurrences (see below). The Shenandoah also has a "riverkeeper" who has catalogued citizen reports of "nuisance aquatic vegetation." The catalogue does not distinguish between filamentous algae and other forms of underwater vegetation, but follow-up investigations by observers trained to recognize filamentous algae in the field could identify river reaches that experience recurring blooms.

B. Recognizing Types of Aquatic Vegetation

It is important for field observers to be able to distinguish filamentous green algae (Chlorophyta) from the other types of aquatic vegetation in streams and rivers. Training and, if possible, certification in the identification of the major plant and algae taxonomic groups will improve the accuracy of nuisance vegetation reports made by volunteers and agency staff.

Filamentous forms of "blue-green algae" (Cyanophyta), which are actually photosynthetic bacteria, are another attached, macroscopic algae found in streams and rivers. Filamentous blue-green algae consist of fine hair-like strands, blue-green in color, that grow to a few centimeters in length and form a slimy coating on the tops of the substrate. They can be important in the nutrient pathways of aquatic environments but generally do not serve as a primary food source like true algae. At high densities, some forms produce toxins known to kill wildlife and domestic animals. Blue-green taxa can multiply rapidly in nutrient-rich conditions and form extensive blooms that break loose from the bottom and float to the surface, where they can be seen as decomposing clumps of scum. They can be found year-round in eutrophic waters.

Periphyton, which are mixed communities of attached bacteria, algae, fungi and other taxa, coat the surfaces of submerged substrates and usually do not exceed a centimeter in thickness. They can serve as an important food source for invertebrates, amphibians, and some fish (Barbour et al, 1999). Filamentous green algae are technically part of the periphyton community where they attach to the substrate but their long filaments stretching into the water column should be considered separately. In this report, the term periphyton excludes filamentous green algae.

Submerged aquatic vegetation (SAV) is a group of vascular plants that live and grow underwater. Sometimes called "underwater grasses," they have evolved special adaptations to the aquatic environment, including aerenchyma (specialized, thin-walled cells with large intercellular air spaces) for buoyancy, thinner leaves and stems, and no waxy cuticle covering on their leaves. SAV inhabit shallow areas where underwater light is sufficient for photosynthesis. They are a food source for waterfowl, provide habitat and refuge to invertebrates and fish, and are a substrate on which algae and other microorganisms can attach.

In addition to filamentous green algae, numerous and varied types of other algae and aquatic plants were encountered at Shenandoah sites during this pilot study: globulose attached algae (e.g., *Nostoc*), attached filamentous cyanobacteria (e.g., *Planktothrix*), beds of aquatic grasses including wild celery (*Vallisneria*), hydrilla (*Hydrilla verticillata)*, water stargrass (*Heteranthera dubia*), and various pondweeds (*Potomageton*), and large colonies of green freshwater sponges. See **Figure 2** for examples.

Figure 2 Underwater photographs of A) cyanobacteria (likely from the family Oscillatoriaceae), B) freshwater sponge, and C) filamentous green algae. Water stargrass, an SAV, appears in every frame. All of these pictures were taken in the South Fork on September 16, 2014.

C. Identifying Waters That May Be Susceptible to Bloom Formation

The stations of a state's water quality monitoring program will not necessarily coincide with the locations of filamentous algae blooms, but water quality data collected at these stations during the algal growing season can be used to identify water chemistries that make blooms more likely to occur. Total alkalinity and water hardness in particular can alter the availability of phosphorus (P), an essential nutrient for algal growth, and make rivers more or less susceptible to blooms. WVDEP staff observed that blooms are more likely to form in West Virginia rivers when alkalinity is greater than 30-40 mg/liter and/or when water hardness is below 120-150 mg/liter (Sommers 2009). Based on a literature review, they reason that, while high calcium (Ca) and magnesium (Mg) concentrations ("hard" waters) suppress algal growth by locking inorganic P into insoluble salts and thus reducing its availability to algae, low Ca and Mg concentrations have the opposite effect of making P more available. High levels of alkalinity, which are principally bicarbonate ions (HCO_3^-), associate strongly with Ca and Mg ions and can further enhance P availability to filamentous algae. Knowing the water chemistry allows field personnel to better anticipate bloom formation in different rivers each year.

The 2015 VADEQ Monitoring Plan identifies twelve Virginia ambient water quality monitoring stations in the Shenandoah rivers: two on the Shenandoah mainstem, four on the North Fork, and six on the South Fork. The stations are visited monthly. VADEQ's probabilistic monitoring program randomly samples macroinvertebrates, habitat, and water quality once a year in Virginia Shenandoah streams. Analysis of these data, possibly in concert with data from neighboring West Virginia, Maryland, and Pennsylvania, could refine and extend the WVDEP findings for identifying flowing waters susceptible to filamentous algae blooms.

D. Site Selection

Filamentous algae growth in rivers and streams tends to recur in the same locations, but the amount of algal growth can differ from year to year. Nutrient enriched river sites often experience blooms, but the blooms can break up or shift in response to flow and weather patterns. Reconnaissance (above) can identify locations in streams and rivers that typically experience filamentous algal growth. If a monitoring program to track filamentous algae is deemed necessary, fixed stations can be established at the locations where algae usually occur. Hence, the monitoring program will have a targeted sampling design. This design can be adaptive. The WVDEP filamentous algae monitoring program provides an example of this. No filamentous algae were observed at the two West Virginia monitoring sites on the Shenandoah mainstem during routine visits, indicating that particular reach of the river is not susceptible to blooms of this type of algae, and monitoring for algae was discontinued in 2014. Ambient water quality monitoring still continues at these two sites, so filamentous algae will be noticed if they appear there in the future. New algae monitoring sites were added in 2014 to the South Branch Potomac River where filamentous algae had been observed in previous years.

E. Timing of Sampling

Filamentous algae may be present throughout a given year when conditions permit, but their abundances generally increase in late spring and again in late summer (e.g., Klapproth and Johnson 2000, Wehr and Sheath 2002, personal observations of ICPRB and WVDEP staff). In the Potomac drainage, filamentous algae blooms typically have declined or disappeared by early October (e.g., Griggs *et al.* 2013, 2014). A sampling season between May and September should be adequate for detecting filamentous algae growth in most Mid-Atlantic rivers. Filamentous algae are vulnerable to scour by flushing events, so persistent high flows in spring or a late summer thunderstorm may temporarily suppress or wipe out algal growth from known hotspots. Field surveys should avoid following moderate to major precipitation events. When the objective of a monitoring program is to quantify the size of an actual algal bloom, sampling can be triggered by reconnaissance findings and should coincide with periods of peak algal growth and abundance.

F. Wadeable Versus Non-Wadeable River Reaches

The methods that can be employed to measure filamentous algae will depend on the water's width and depth at the monitoring site. Certain sites may be wadeable during summer low flows and non-wadeable other times of the year. Precipitation events may cause conditions hazardous for wading, and wadeable surveys are never appropriate under flood conditions.

The sections of the Shenandoah mainstem and the Forks suitable for wadeable methods and those that will require non-wadeable methods are listed in **Appendix E**. The sections are equivalent to the 43 Virginia assessment units (AUs) assigned to the Shenandoah mainstem and the Forks. The AUs range in length from 1.15 km to 30.8 km. The minimum and maximum width of each AU was determined using desktop mapping (aerial imagery from Google Earth). A river width of 100 meters was used as a distance at which transect surveys begin to become unwieldy and too time-consuming. Water depths vary longitudinally, changing as riffle and runs empty into pools. No one section can be assumed to be wadeable for its entire length, but professional experience and a familiarity with the river sections helped to identify likely wadeable and non-wadeable sections.

6. Field Sampling Methods

The first objective of this pilot project was to develop quantitative, repeatable, and scientifically valid methods for measuring filamentous algal growth using the Shenandoah River basin as a study area. The WVDEP lateral transect method served as a starting point. The method, which has been used by WVDEP for several years, is designed to estimate percent algal cover along a cross-sectional, or lateral, transect line in streams and wadeable rivers. The method, as typically implemented by WVDEP, is not easily performed in rivers deeper than 1 m or wider than 100 m. It produces estimates of algal coverage across a single, lateral transect, usually at the point of maximum algal cover, and is not designed to measure the areal extent of a bloom. The scope of work for this pilot study proposed adapting the WVDEP method to a) estimate percent algal cover along a lateral transect in non-wadeable rivers, where depths greater than 1 m make algal measurement from a standing position impractical, and b) document the occurrence of algal patches along a longitudinal transect, or river reach, by performing lateral transects at the points on the longitudinal transect where algal patches are encountered. In the course of the study, a fourth method was developed: a longitudinal protocol using an algal subsampling approach. The four methods (**Table 1**) allow observers in the field to estimate percent algal cover rapidly, for several purposes, in all of the stream and river types experienced in the Shenandoah River basin. The methods are described in detail in this section.

Table 1 Filamentous algae methods investigated for different rivers types and applications in the Shenandoah River system.

River Type	Station Applications	Reach Applications
Wadeable	WVDEP Lateral Transect Method	
Non-Wadeable	Adapted WVDEP Lateral Transect Method	
Wadeable and Non-Wadeable		Longitudinal Protocol using Lateral Transect Methods
Wadeable and Non-Wadeable		Longitudinal Protocol using Algae Subsample Method

A. The WVDEP Lateral Transect Method

In 2007, The West Virginia Department of Environmental Protection (WVDEP) began receiving complaints of filamentous algae interfering with recreational uses in certain rivers of the state. In response, WVDEP develop the lateral transect method and worked with a consultant to perform a survey of recreational users. In the survey, respondents answered questions about their uses of West Virginia rivers and their experiences with filamentous algae, and reviewed WVDEP photographs of algae to indicate the levels of algal cover that would dissuade them from fishing, swimming, or boating. From these responses, WVDEP determined a threshold of algal cover in a lateral transect that was acceptable for recreational uses, and promulgated numerical criteria to assess which rivers no longer supported recreational uses.

Algal cover in a lateral transect that exceeds an instantaneous acute criterion of 40% was established as a violation of West Virginia recreational uses. Algae cover between 20% and 40% also violates recreational uses if found in three (3) separate transects of an algal patch, and the algal patch extends in length at least three times the average channel width at the site. WVDEP understands that their monitoring program likely misses the full extent of algae blooms, given the patchiness of the observed distributions and the algae's boom-and-bust growth cycles. The state therefore focuses on measuring filamentous algae where it is encountered and uses those measurements to evaluate larger, contiguous river segments, resulting in listings covering areas beyond just the observed area.

WVDEP Protocol

A detailed description of the WVDEP method can be found in West Virginia's Standard Operating Protocol (**Appendix C**). A shore-to-shore transect representative of the general site location is established, typically at the onset of an algal bloom, and the same transect is used on repeat visits to monitor change in algal growth at the site during the year. A transect is often placed upstream of a riffle, in a run or glide area, because that is where filamentous algae tend to be found. The GPS coordinates at the transect ends are recorded at each bank. Care is taken to avoid disturbing the algae prior to the measurements. If algae are present only in trace or low amounts, a single cover estimate is made of the entire transect. Similarly, if algae are present at levels above 80%, only a single cover estimate is required. Intermediate amounts of algae require visually dividing a transect into five or more homogeneous segments, so that separate estimates can be made of the individual segments. It is assumed that visual estimates of field observers are highly accurate at very low and very high algae abundances, whereas estimates of moderate algal abundances are improved by examining multiple, smaller, more homogeneous segments. Segments along the transect line are delineated by noticeable changes in channel geomorphology, algae type, and SAV, in order to reduce the variability within a segment. Estimates are made in a 1 m wide strip under the transect line. The field observer estimates and records the percent of stream bottom in each segment strip that is covered by filamentous algae growth. "Covered" means that filamentous algae are present—either attached to the bottom or in the water column—and are visually obscuring the stream bottom.

The materials required to perform the protocol include: 100 m tape measure (transect line), measuring staff, camera, and the WVDEP Filamentous Algae Monitoring Form. The form is included for reference in **Appendix D**. Page 1 of the monitoring form records typical site and sample information such as time and coordinates of the sample. Page 2 captures much of the qualitative data on SAV, filamentous green algae (FGA), relative abundance, water chemistry, weather, and notes, etc. Page 3 is the algae transect measurement form, where detailed data describing the transect area is recorded, and page 4 captures landowner and photographic documentation.

In the WVDEP SOP, a width of approximately one (1) meter around the transect line is evaluated as field observers move from one bank to the other. ICPRB staff found that a larger area of one meter on each side—up to five meters—can be evaluated in the lateral transects with no loss of precision in the field observers' algal cover estimates.

FINDINGS

The WVDEP lateral transect method provides a rapid assessment of the general conditions at the site; estimates of percent cover by filamentous green algae, blue-green algae, and SAV along the transect line; and a description of the overall physical habitat conditions. ICPRB staff have implemented the method in West Virginia tributaries to the Potomac River mainstem for three field seasons. A two (2) person sampling team is capable of monitoring between six and ten sites per day, including travel to and between locations. The method, including collection of water chemistry, can be completed in as little as 15 minutes per site if no algae are present, with an additional 15 – 60 minutes per site if a bloom requires measurement. Additions have been made to the West Virginia protocol since the program's inception, including recording on the field form the substrate type, the percent cover of SAV along the transect, and quantitative measurements of tree canopy cover. The method is incorporated easily into other biological or water quality monitoring programs.

B. ADAPTING THE WV LATERAL TRANSECT METHOD TO NON-WADEABLE SITES

The WVDEP lateral transect method for measuring filamentous algae at wadeable sites can be applied to non-wadeable sites, using canoes or other small boats to navigate the deep water. This adaptation is not employed very frequently, as algae occur less abundantly in deeper waters, and wide rivers can make collection of cross-sectional measurements difficult. It should be noted that a certain skill in handling canoes or small boats is required to navigate a straight transect across a non-wadeable channel.

PROTOCOL

A bank to bank, lateral transect is established as per the wadeable method in a section of river where filamentous algae are present. Instead of a measuring tape, a laser range finder can be used to measure transect segment lengths and positions by shooting back to the bank where the transect starts. Transect segments are again delineated by changes in channel geomorphology, algae type, and SAV. If encountered, contiguous sections of algae or SAV should be broken into transect segments of 10 m or less, in order to maintain comparable estimates of algal cover. All other aspects of the lateral transect method are performed as usual. Algal measurements at the site can be made with a combination of the wadeable and boatable techniques because the transect segments are established in the same manner. The WVDEP Filamentous Algae Monitoring Form (**Appendix D**) can be used as-is to record estimates from non-wadeable sites.

In canoes, a technique performed by the rear paddler, known as "ferrying," is usually successful in staying on a straight transect while the biologist in the bow of the canoe records the observations, depths, and takes documenting photographs. Ferrying involves setting a slightly upstream bow direction that allows the paddler to paddle at a steady pace and the "line" of the canoe offsets the river current, allowing for a straight transect across the channel.

FINDINGS

Although designed for a single, monitoring site with a non-wadeable channel, this protocol actually was tested during the longitudinal surveys (described below) performed for the pilot study. It proved successful when it was implemented in non-wadeable rivers with few or no rock ledges and large boulders.

C. ADAPTING THE WV LATERAL TRANSECT METHOD TO LONGITUDINAL SURVEYS

One purpose of longitudinal surveys can be to find and rapidly evaluate maximum levels of filamentous algae growth in river segments. This method is especially useful in those parts of a river that are not readily accessible. WVDEP currently uses an *ad hoc* approach to implement this type of survey. Their longitudinal surveys often begin and end at public access points, in order to evaluate reaches of recreational importance. The surveys are usually performed from boats or canoes, but can be completed from vehicles where proximal roads allow for a mostly continuous view of the stream or river. When algae are encountered in wadeable waters during a longitudinal survey, estimates are made using the WVDEP lateral transect method. As of summer 2014, longitudinal surveys had not yet been standardized and lacked specific field forms and operating procedures. For the Virginia Shenandoah pilot study, ICPRB biologists incorporated the WVDEP lateral transect method for streams and wadeable rivers, and the adapted method for non-wadeable rivers, into a longitudinal protocol. ICPRB biologists performed trial runs of the protocol on the Shenandoah North Fork and South Fork. Biologists from WVDEP were consulted during the development of these protocols. They tried the pilot protocol on the South Branch Potomac River in West Virginia and provided feedback on their experience.

PROTOCOL

A longitudinal protocol is performed when incidental observations or other supporting information suggest a river reach is experiencing filamentous algae blooms. A portion of the river suitable for a longitudinal survey is determined first. This can be based on known access points and aspects of the river's size and condition. In order to make the results of these surveys more transferrable to other programs, the length of the reach to be surveyed can be determined in a manner similar to that of EPA's Environmental Monitoring and Assessment Program (EMAP) and National River and Streams Assessment (NRSA) programs. In those programs, the length of an assessment unit is set to a value equal to 40 times the average channel width of the stream or river at the site, and up to a maximum length of four kilometers. Four kilometers is also a manageable distance for a day's survey, given travel and preparation time. Alternatively, longitudinal protocols can be performed on river reaches corresponding to a state's assessment units.

The WVDEP lateral transect method for streams and wadeable rivers, and the adapted method for non-wadeable rivers, are deployed at intervals during the longitudinal surveys. Lateral transect points can be established in regular or random intervals, when algae are encountered, or when river morphology changes. WVDEP currently establishes transects where the maximum cover of a distinct algae patch is encountered, in order to determine if percent algal cover fails the West Virginia's filamentous algae criterion (see above).

For demonstration purposes, a longitudinal protocol similar to that of WVDEP was tested in this pilot study, with incidental measurements made at cross-sections exhibiting maximum algae cover. The river reach was divided into longitudinal segments when significant changes occurred in the dominant plant communities or in river geomorphology. The first segment began at the access location. GPS coordinates were taken at the beginning of each new segment, and the GPS unit's measure of error (EPE) was recorded on the field form (**Figure 3**). Segments with noticeable algae cover were measured with the WVDEP lateral transect method, noting the segment and coordinates where the measurements took place. The measurements were performed by wading if possible, or from boats in non-wadeable waters.

As field observers downstream, they qualitatively documented aspects of the river's habitat and plant and algae community. Qualitative abundance of different types of algae, and plants are rated 0 – 4 (0 = Absent, 1 = Rare, 2 = Common, 3 = Abundant, 4 = Extreme). In addition, the location and structure types of any filamentous algae encountered were recorded.

Data were recorded on the algae transect measure field sheet, unmodified from page 3 of the WVDEP Filamentous Algae Monitoring Form (**Appendix D**). The lateral transect was divided into segments in the field, per the WVDEP SOP. A laser range finder with an accuracy of at least 0.1 m was used to establish segments along the lateral transect, and GPS coordinates were taken at each end of a transect. (ICPRB staff noted that laser range finders greatly increase the ease and speed of delineating the lateral

Stream Name													
Site-Section ID						Date		Time		Geo		Bio	
GPS type			GPS EPE			Plants/Algae (0-4)		Pic DSCN#	Picture Subject/Notes				
Start Lat					N	FGA							
Start Long					W	BGA							
Finish Lat					N	MOSS							
Finish Long					W	PERI							
Filamentous Algae		Algae observed in section (Enter " √ " for all that apply)				Emerg AV							
< 1 %	E	**Location**		**Structure**		Float AV							
< 5 %	E	Channel		Periphytic		SAV							
5 – 10%	E	Littoral		Globular		Total Plants							
10 – 40% (3 meas.)	M	Shallows		Benthic mats		**In-situ water quality meter.**							
	M	Pools		Benthic "Tufts"		WTEMP (°C)		**Notes:**					
	M	On SAV		Surface mats		pH (SU)							
40 – 80%	M	Attached		Filaments < 1 m		DO (mg/L)							
80% +	E	Floating		Filaments > 1 m		COND (µmhos/cm)							

Figure 3 The pilot longitudinal transect method segment field form.

segments compared to traditional tape measures.) Canopy cover readings were recorded using a densiometer, an approach borrowed from EPA's EMAP program (Angradi 2006, Flotemersch *et al.* 2006). Estimates of filamentous algae cover in each segment were arrived at, through consensus, by two field observers viewing the same section of the transect.

Method Demonstration

The pilot protocol was tested on both the North Fork and South Fork in early September, and was found to be reasonable for application in the North Fork test area, from Deer Rapids to Strasburg.

Figure 4 Documenting very high filamentous algae cover on the North Fork using the lateral transect method in a longitudinal survey.

The North Fork survey was performed on September 15, 2014, after receiving citizen reports of algae growth. Three ICPRB biologists in two canoes traveled from Deer Rapids to Strasburg, recording algal estimates and observations along the river reach. Algae was observed almost immediately from the put-in, and gradually increased in percent cover, quickly reaching 40%. When the algae patch ended, a second segment was established. When algae reappeared in the river channel, segment 2 ended and segment 3 began, and so forth. Eight segments were delineated during this survey. In each, algae was measured using the WV lateral transect method when called for by the protocol. Algae cover ranged from zero to greater than 90% (**Figures 4** and **5**). In

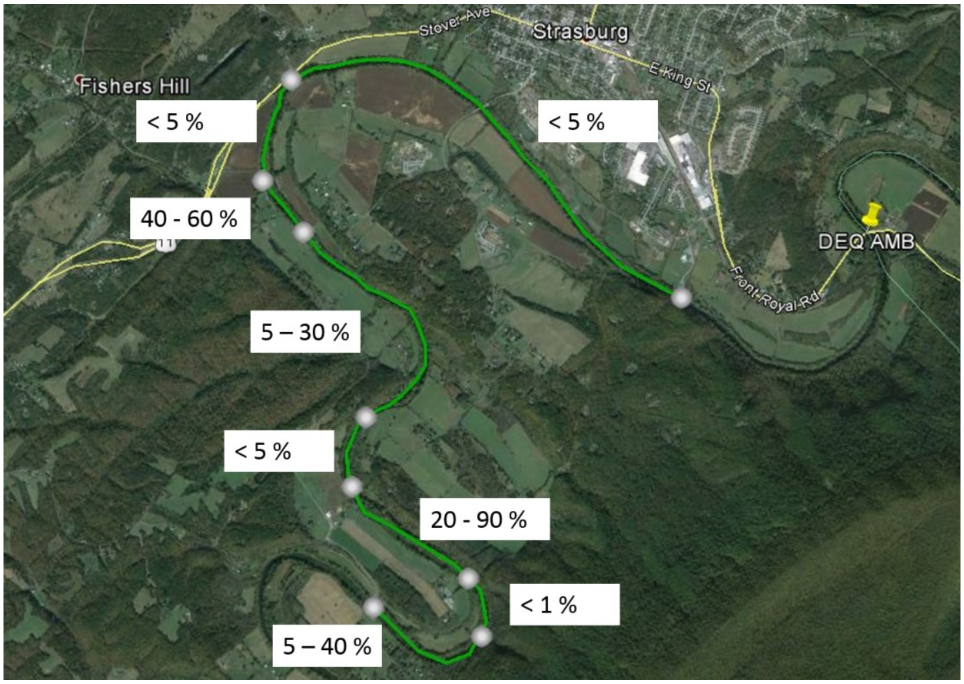

Figure 5 Section of the North Fork sampled with the longitudinal incidental transect method. In this method, observed changes in algae and plant communities denote the segments, changing at the locations of the grey dots. Estimated and measured algae cover percentages are shown next to each segment. A VADEQ ambient monitoring station is located just downstream of the sampled reach.

segment 3, algae cover measurements of 19.9%, and 40.52%, and >90% were recorded as algae cover increased. Algae ended abruptly at a riffle at the start of section 4, but ranged between 5% and 60% in the next two segments. In between algal patches, aquatic vegetation or open river bottom dominated. SAV included water stargrass and an unidentified sago pondweed/riverweed. Numerous large freshwater sponge colonies were also observed, and the last river segment was dominated by *Chara*. The patchy nature of the algae and vascular plant distributions observed on the North Fork that day match numerous experiences of ICPRB staff on other rivers in the region. The survey field data are included in **Appendix F**.

The protocol was also tested on the South Fork, between Shenandoah State Park and Karo Landing, on September 16, 2014. The WVDEP lateral transect methods were found to be impractical at this location, even in boats, due to the South Fork's wide, shallow channel and the presence of river-wide, diagonal rock ledges and riffles. Several transect measurements were made from boats, using laser range finders to determine transect segment lengths. Even for practiced canoeists, maintaining a straight line of travel across a lateral transect in flowing waters was difficult, and ultimately preoccupied the crews as they tried to assess and measure algal abundance. The South Fork experience highlighted some of the physical impediments that can make lateral transect estimates impractical in large rivers.

FINDINGS

Longitudinal surveys using the WVDEP lateral transect method in streams and wadeable rivers, and the adapted lateral transect method in non-wadeable rivers, are useful for observing and quantifying instances of filamentous algae in inaccessible reaches of small to medium sized rivers. The method is

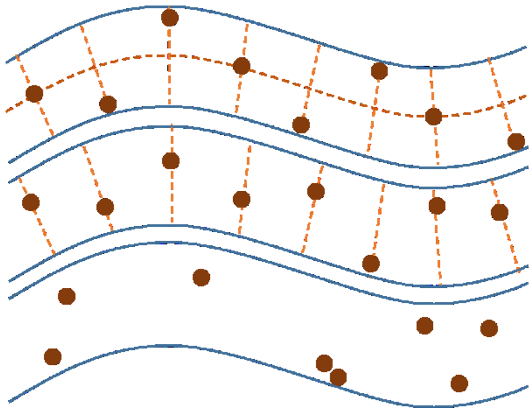

Figure 6 Diagram showing the difference between stratified (top), stratified-random (middle), and pure random (bottom) sampling.

time intensive and not practical for routine use. It should be employed in areas of known or suspected filamentous algal blooms, during peak growth seasons, and is best used to determine if instances of algae growth exceed established screening thresholds.

D. Developing a Subsampling Method for Longitudinal Surveys

Another purpose of a longitudinal survey may be to quantify the areal extent of filamentous algae cover in an entire segment, or river reach. Given this purpose, measurements of percent algal cover made with a random or grid sampling design are more appropriate than the incidental measurements of the previous longitudinal survey method. The sometime difficult, time-consuming task of performing lateral transect measurements as part of a longitudinal survey in a large river, and interest in a method that estimates algae cover for an entire river segment, prompted the development of this subsample-based pilot protocol. The method is scalable to different segment sizes, able to be performed over large areas relatively quickly, and accommodates both random and grid sampling designs. It can address the question: "what percent of an entire segment is covered by filamentous algae?" The method remains analogous to the WVDEP lateral transect method and two adapted methods (above) in that percent algal cover is still quantified with visual estimates made by field observers; however, lateral transects are replaced with sampling points located throughout the segment.

Protocol

A longitudinal survey using the subsampling method is initiated and planned in the same manner as the longitudinal survey using the lateral transect method (above). The number and location of subsampling points in a segment will depend on the choice of sampling design, the statistical confidence required in the estimate of percent algal cover, and the amount of staff effort deemed appropriate for the task.

Designs for subsampling a river segment can be stratified (which is essentially a grid), stratified-random, or entirely random. Examples of each design are shown in **Figure 6**. A stratified design ensures proportional coverage of the river segment's length and cross-sections, and is the recommended approach unless an assessment program requires a random design. The stratified design is similar to the designs used in the EPA NRSA and EMAP programs in that it proportionally samples sites across a river's channel as the observers move downstream. In the pilot study's trial run of the protocol, a stratified design was implemented, with sampling points located every 100 m along the river's length, rotating between five cross-sectional locations (i.e., right, center-right, center, center-left, and left).

During the field trials, the percent of algae covered bottom area of an approximately 10 m diameter circle is evaluated at each sampling point. A 10 m circle was selected through trial and error and best professional judgment as an effective area to evaluate, with no loss of visual estimate precision. Field observers estimate the percent cover of filamentous green algae, blue-green algae, and SAV inside the 10 m circle. It should be noted that the percentages are independent of each other, and do not have to total 100%. For example, abundant SAV beds may cover 90% of the observed bottom, but filamentous green algae have colonized 50% of the SAV, making the sum of the individual totals more than 100%.

Implementing the longitudinal surveys requires little more than the standard equipment usually employed by field biologists to sample rivers. The protocol requires a GPS unit programmed with coordinates of the sample locations, measuring staff, laser range finder, waterproof camera, datasheets and a writing implement. Boats—either canoes or shallow drafting jon boats—and safety gear are needed to access non-wadeable sections of river. River access locations, including public boat ramps, will likely determine longitudinal routes within suspected algal areas or assessment units. A field datasheet developed for this method is provided in **Appendix G**.

METHOD DEMONSTRATION

On September 24, 2014, three ICPRB biologists tested the subsample method in a longitudinal survey of a segment of the South Fork. ICPRB staff had earlier found the WVDEP lateral transect methods to be impractical at this location due to the South Fork's wide, shallow channel and the presence of river-wide, diagonal rock ledges and riffles (see above). River access points determined the area of investigation, between the Shenandoah State Park and the Karo Landing at the mouth of Gooney Creek. The section is entirely within the lower end of Virginia Assessment Unit #303 (**Appendix E**).

A stratified sampling design was employed, in keeping with a NRSA-type demonstration of the protocol, and sampling coordinates were determined before the survey. The central coordinates of each transect were located every 100 meters along the river's length, starting at the access point. Forty (40) sampling coordinates were determined, resulting in a longitudinal river length of approximately four kilometers. As observers move downstream, sampling points rotated through five lateral positions: left bank, 25% (left of center), 50% (center), 75% (right of center), and the right bank.

Observers 1 and 2 were in the same boat and made separate algal cover estimates, at the same time, of each sampling location, resulting in sample duplicates for each location. Observer 3, following in the second boat, made an estimate of the same sampling locations. His estimates more closely resemble replicate samples because they were done at a different time and from a different boat.

As the observers arrived at each predetermined sampling coordinate, observer 1 would stand and survey the surrounding river bottom in a 10 m diameter circle for filamentous green algae, cyanobacteria, and aquatic vegetation while observer 2 held the boat in place. Once observer 1 recorded his observations and percent cover estimates, observer 1 and 2 switched roles and observer 2 made a visual assessment of the same area. When they were done and had left the sampling area, observer 3 approached the sampling coordinates and made his observations. Some variability in the sampling areas was introduced since observer 3 navigated independently to each of the sampling coordinates.

In addition to algae and plant estimates of percent cover, a representative depth was recorded at each sampling location, along with the dominant substrate, field coordinates, the time, and a representative photograph of the surveyed area. The percent cover estimates from the 40 coordinate sample areas and the three observers can be found in **Appendix H**.

The data from the three observers was averaged over the 40 subsamples for FGA, BGA, and SAV, both independently and as an average of the observers' ratings in order to estimate total coverage of the surveyed reach. Average percent cover of SAV ranged between 20.38% and 25.13% for the three observers with a mean of 23.14%. Filamentous green algae measured between 17.98% and 21.33% among the three observers, with an average of 19.99%. Cyanobacteria ranged between 3.45% and 8.55% with an average measure of 6.72% among the 40 sites. Confidence estimates were also calculated, and were broad at the 95% confidence interval, owing to the patchy nature of the algae observed (**Table 2**). The confidence interval (CI) of the rater-averaged estimate of filamentous green algae cover ranged between 11.96% and 28.02%. The aquatic vegetation CI was somewhat tighter,

Table 2 Average percent cover of aquatic vegetation (SAV), filamentous green algae (FGA), and cyanobacteria (BGA), with 95% confidence interval estimates of algal and plant cover in the study reach.

Subject	Observers	Mean	SD	Error	95% Conf. Int.
SAV	Obs 1	23.93	23.21	7.23	16.70 - 31.15
	Obs 2	25.13	25.02	7.79	17.33 - 32.92
	Obs 3	20.38	17.52	5.46	14.92 - 25.83
	Average	23.14	21.30	6.63	16.51 - 29.78
FGA	Obs 1	20.68	27.50	8.56	12.11 - 29.24
	Obs 2	21.33	27.57	8.58	12.74 - 29.91
	Obs 3	17.98	24.11	7.51	10.47 - 25.48
	Average	19.99	25.78	8.03	11.96 - 28.02
BGA	Obs 1	8.15	17.41	5.42	2.73 - 13.57
	Obs 2	8.55	18.24	5.68	2.87 - 14.23
	Obs 3	3.45	6.30	1.96	1.49 - 5.41
	Average	6.72	13.75	4.28	2.44 - 11.00

ranging between 16.51% and 29.78%. All three subjects ranged broadly in their abundance between sampling locations. Individual subsamples ranged from 0% to 98% for SAV, 0% to 90% for FGA, and 0% to 80% for BGA, giving an indication of the variability in abundance from one location to the next. However, SAV was generally present and somewhat abundant in most sites being observed in about 35 of the 40 sites, with 30 of those greater than 5% coverage. Green algae was present at about 34 sites but only above small amounts in 17, and BGA with 23 and 7, respectively.

The data presented above are for a single segment on the day of observation, for three experienced field observers. It should not be assumed that this level of algae was typical of other areas in the South Fork, or that it captured the maximum amount of algae at that location for the season. The same section of river was surveyed in July during pilot algae and photographic method testing, and no filamentous green algae was observed. During that time, decaying Cyanobacteria mats were abundant in the section, often swelling with gases as they respired and decayed, and lifting from the bottom to float to the surface and downstream.

FINDINGS

Longitudinal surveys using the subsample method should be useful in observing and evaluating algae cover in non-wadeable, medium to large rivers. The method can be particularly useful in evaluating inaccessible reaches of a river, and can be done more rapidly than the adapted, lateral transect method. Algae cover can be sampled with a stratified or random design, depending on programmatic requirements. The method should be employed in areas of known or suspected filamentous algal blooms, during peak growth seasons. It is intended to produce a quantitative estimate of % algal cover for an entire river reach or assessment unit.

7. VOLUNTEER MONITORING

The second objective of this pilot study was to develop and test a citizen monitoring program for filamentous algae. The program was intended to teach citizen monitors how to a) correctly distinguish filamentous green algae from blue-green algae and other aquatic vegetation in streams and rivers, and b) apply the appropriate quantification methods if filamentous green algae are observed. This objective was suggested in part due to the number of citizen reports of nuisance plant growth, and the presence of two well-organized and long-standing citizen monitoring organizations in the basin.

A. TRAINING

Conversations and scoping meetings took place beginning in March 2014 between ICPRB and known local Shenandoah citizen watershed organizations, including the Friends of the Shenandoah River (FoSR) and the Friends of the North Fork of the Shenandoah (FNFSR). Early discussions between FoSR and

FNFSR members were positive, enthusiastic, and full of questions about the pilot study and its scope. The meetings raised interest among the two organizations' established citizen monitors and other non-monitoring members alike, but also raised concerns about the proposed method, which was based on the WVDEP lateral transect method for wadeable sites. The WVDEP method was adapted for citizen use by eliminating non-essential aspects of the protocol and revising the datasheets accordingly.

A training curriculum was developed during March and April 2014, and a one-day training session was held on May 13, 2014. It was well attended, with approximately twenty citizen monitors and biologists from the Pennsylvania Department of Environmental Protection, VADEQ, and USEPA. WVDEP staff Kevin Coyne was on hand to answer questions about the method's application and use in West Virginia's regulatory process. The training consisted of a half-day of classroom instruction on algal measurement methods and a primer in algae and aquatic plant identification, followed by an afternoon field demonstration and practice session (**Figure 7**). Monitoring equipment for ten teams had been assembled into kits and delivered earlier to the FNFSR offices in Woodstock, VA. Monitoring equipment included waterproof cameras, measuring tapes, measuring rods, and waterproof datasheets. Citizen monitors were invited to either monitor their already established sites, or two other sites where they would comfortable applying the wadeable methods.

B. 2014 Volunteer Results

The anticipated participation in the pilot volunteer monitor program during the summer of 2014 was not realized. Several factors contributed to the eventual lack of monitoring observations made by the volunteer monitors. First, many of the training attendees did not feel entirely comfortable performing the in-stream protocol, which can involve wading in sometimes swift water on slick river beds. Second, the work requires teams, which complicated the logistics of volunteer monitors who are accustomed to individually visiting their water quality monitoring sites. Third, some of the individuals who expressed interest in the program were unable to make the training date. Fourth, there were issues of consistent information exchange between ICPRB, the volunteer monitor coordinators, and the monitors themselves. The ICPRB biologists assumed that the established monitoring programs would be able to implement the protocol with minimum oversight. ICPRB staff were frequently in the field collecting data for other projects, making communications with the volunteers difficult. Fifth, monitors changed over time, with some dropping out and new ones, who needed training, coming forward. Lastly, there was a

Figure 7 Citizen monitors and state agency biologists gather at the Friends of the North Fork offices for training in the West Virginia Filamentous Algae Protocol and to practice the method in Stony Creek, Edinburg, VA.

significant amount of time that passed between the May training session and manifestation of the algae blooms in the Shenandoah. The blooms peaked in late August and September of 2014. This undoubtedly contributed to a loss of enthusiasm for the project. Lessons were learned and this part of the pilot project could be improved in the future.

Volunteers should first and foremost be able to recognize the different plant types found in Mid-Atlantic streams and rivers. Citizen monitor organizations seeking to monitor filamentous algae should also have a state approved Quality Assurance Project Plan (QAPP) and receive training in river safety. Volunteers have already demonstrated they can serve as “eyes on the river,” and if trained individuals have a reliable way of reporting occurrences of filamentous algae, they can provide much of the reconnaissance need in a filamentous algae monitoring program. Performing the WVDEP lateral transect method in streams and wadeable rivers can be done safely by the most capable volunteers, but should not be required of all volunteer monitors. Volunteers should monitor river sites in teams, for ease and added safety. The longitudinal survey methods present more challenges, but could also be performed by trained individuals capable of meeting the methods’ physical demands.

One benefit of a volunteer monitoring program for filamentous algae could be a more nuanced understanding in the community of aquatic vegetation. It was apparent in this pilot project’s May training session that, before the session, some attendees did not distinguish between the various plant types, were only vaguely aware of the role of plants and algae in the aquatic ecosystem, and tended to classify all underwater vegetation as “bad.”

8. Precision of Visual Estimates in the Field

Some variability in the visual estimates of percent cover is to be expected, and decisions need to be made about acceptable levels of variance. Field observers should receive training in visual estimation techniques, and practice the techniques with experienced staff, before making actual measurements. Trained observers can estimate algal cover independently, which provides a measure of the variability between individuals, or may discuss their estimates at each site and determine a single value by consensus. Field observers may find that their estimates are improved by considering half or a quarter of the circular area or transect strip at a time, instead of trying to visualize and asses the entire area to be evaluated. Photographs showing river sites with a known algae cover, such as those provided in the WVDEP SOP (**Appendix C**), can help an observer refine his/her estimation techniques.

A statistical analysis was performed on the independent visual estimates of percent algae and plant cover made by three trained observers during the South Fork Shenandoah method demonstration on September 16, 2014. The comparisons provide a baseline for the degrees of variability that can be expected in trained observers. Visual estimates were made at forty sampling points located in a previously defined segment of the river. Results are summarized in **Table 2** and further analyzed here.

Statistics of observer, or “rater,” consistency (reliability scores) were calculated on the percent cover estimates recorded by observers 1, 2 and 3 for filamentous blue-green (Cyanobacteria) algae, filamentous green algae, and SAV (**Table 3**). Specifically, intra-class correlation coefficients (ICC) for quantitative data were employed in a one-way model of inter-rater consistency at the 95% confidence level using the “irr” package in R (Bartko 1966, Hallgren 2012, Gamer 2012). The

Table 3 Inter-rater reliability scores for the three field observers (n = 40). ICC, intra-class correlation coefficients.

Subject	ICC	p-value	95% confidence limit
Cyanobacteria	0.736	<<0.001	0.607 - 0.837
Green Algae	0.926	<<0.001	0.880 - 0.957
SAV	0.885	<<0.001	0.818 - 0.932

statistic calculates a relative coefficient of agreement between the raters. Inter-rater reliability (IRR) is considered poor for ICC values less than 0.40, fair for values between 0.40 and 0.59, good for values between 0.60 and 0.74, and excellent for values between 0.75 and 1.0. Overall, inter-rater consistency between the three observers in this demonstration of the method was very high: 0.736 for cyanobacteria, 0.926 for filamentous green algae, and 0.885 for aquatic vegetation. Observers 1 and 2 had even higher ICC scores when tested without observer 3, which is likely a result of the aforementioned replicate versus duplicate nature of the three sets of observations. A one-way analysis of variance (ANOVA) was also performed on the data collected by the three observers for each plant types, and no significant differences were found in the observer estimates. While the precision and repeatability of the visual estimates rely on the abilities of the field staff, the data demonstrate that experienced staff can make fairly reliable, repeatable estimates of percent algal cover.

9. Field Photography and ImageJ Analysis

It might also be possible to verify visual estimates with digital, image processing software such as ImageJ. For this pilot study, the ImageJ software was applied to digital images showing different levels of algal cover in the field. The application's usefulness as a tool for independently verifying visual estimates of algal cover was explored, and a better understand was gained of the potential of image analysis software to quantify algal cover and reduce possible observer bias. Pilot study investigators also wanted to quantify the inherent variability in the algal cover estimates made by observers. Six individuals evaluated eight photographic images of algal cover taken in the field, and the results are discussed below. Finally, the image processing and analysis steps identified strengths and limitations of the ImageJ software.

A. Field Photography

Field-captured, digital photographs were acquired using a Nikon AW100 camera with and without a circular polarizing filter. Images were taken in the window of time between 10:00 - 16:00, in both submerged and above water environments, and in a variety of weather conditions. Areas with varying algal abundance, water clarity, and species composition were targeted. A color standard, DGK Color Tools WDKK Waterproof Color Chart, was used in field captured photographs as a color reference.

Capturing high quality images of a specific sampling area in the stream or river proved critical to obtaining useable digital images for estimate verification. Contrary to photographing an object indoors, where external elements such as incidental light intensity, color calibration and angle of shot can be controlled, field acquired images are subject to the ambient light environment. The three image-capture perspectives in the field—shore-side, near field above surface, and submerged—come with different advantages and limitations that need to be considered.

A near field image taken directly above the water's surface of an algae covered area is capable of producing an image that can be quantified using ImageJ analysis. For image captures of this kind, a circular polarizer reduces glare and creates a clearer image. Further glare reduction is possible when a shadow is cast across the area of interest. Shaded areas are often created by overhanging trees or river banks. In scenarios when there is a lack of overhead cover, shaded areas can be artificially created using a dark colored sunscreen. Multiple images should be taken of the same region to better the chances of an image with minimal distortion. Images acquired from above the water surface are easily affected by wind, rain, and other extrinsic factors and therefore should be taken only when ideal conditions are met. Water clarity is a critical factor in the acquisition of a useable image. ImageJ relies on differences in hues to distinguish algae from substrate, and not the hue itself. In highly turbid waters, the observer's ability to differentiate between substrate, submerged aquatic vegetation, and filamentous algae becomes impaired. The ImageJ protocol should not be used if water turbidity is moderate or heavy.

Images captured with a submerged camera have the highest potential for producing ImageJ estimates of algal cover. The images are not affected by glare and do not have surface distortion. The water surface acts as a polarizing light filter, so underwater shots do not need additional hardware (i.e., polarizing filter). The most important light variable for underwater shots is the time of day. The hours around solar noon yield the best images with minimal shadows cast by underwater objects in the sample area. Water clarity is again a crucial factor in successfully capturing underwater images. Submerged images are quickly compromised by suspended particulates which influence the image unevenly depending on distance from the lens and size of the particle. It is the opinion of ICPRB staff that when water clarity and time of day requirements can be met, underwater image capture is the preferred method if digital image analyses are planned.

B. Image Processing

To analyze the field-captured digital images, the 64-bit version of ImageJ was downloaded onto a PC running Windows 7 Professional. ImageJ is open source, multi-platform (Windows, Mac, and Linux interfaces in both 32-bit and 64-bit), JAVA-based imaging software developed by the National Institute of Health (NIH) to analyze digital images. The free software is able to process a variety of image file formats (TIFF, JPEG, PNG, etc.), and is supported by an extensive library of plug-ins capable of performing a variety of image manipulations, calculations, and tasks. The most current version of JAVA software is needed upon first installation of the ImageJ software, so that plugins and analysis tools work correctly. The plug-in *Threshold_colour* was used to separate and dissect green (algal) hues in each image. The *Threshold_coulour* software, a free plugin, was downloaded from a third party developer site (http://www.mecourse.com/landinig/software/thresholdcolour.zip). Instructions developed by the pilot study staff explained how to identify and isolate algae in the photographic images using the ImageJ technology (**Appendix I**). The application's output is an estimate of the percent area delineated by the user in a series of color, brightness, and hue selection steps.

C. Method Demonstration

The six observers (ICPRB staff) evaluated eight field-captured images of algal cover (**Appendix J**) using a visual estimation method and the ImageJ software application. Prior to using ImageJ, the observers estimated the percent algal cover in each image using their best judgment, without visual reference or aid. The observers then applied the ImageJ application and produced estimates of algae percent cover for the same images (see example in **Figure 8**). The visual- and ImageJ-based estimates were compared using a paired t-test to identify statistical differences. An intra-class correlation coefficient (ICC) statistic was used to identify agreement between users.

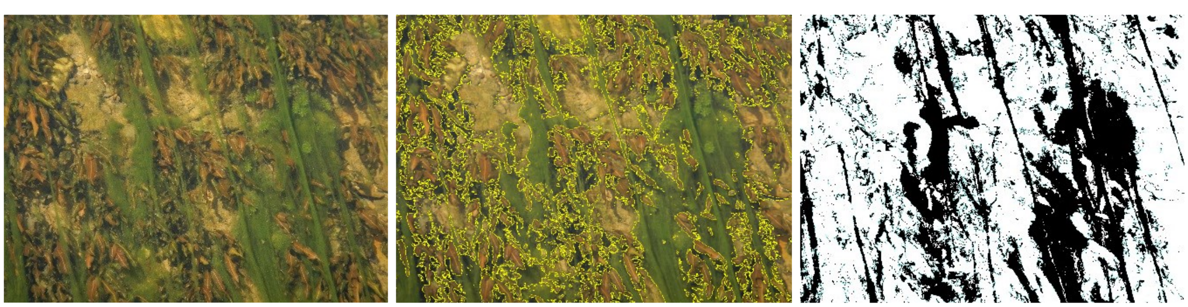

Figure 8 Test image #3 pre and post ImageJ photo processing. ImageJ input image (left), mid-process image (middle) with algal delineations made by observer, and output image of algal area (Right).

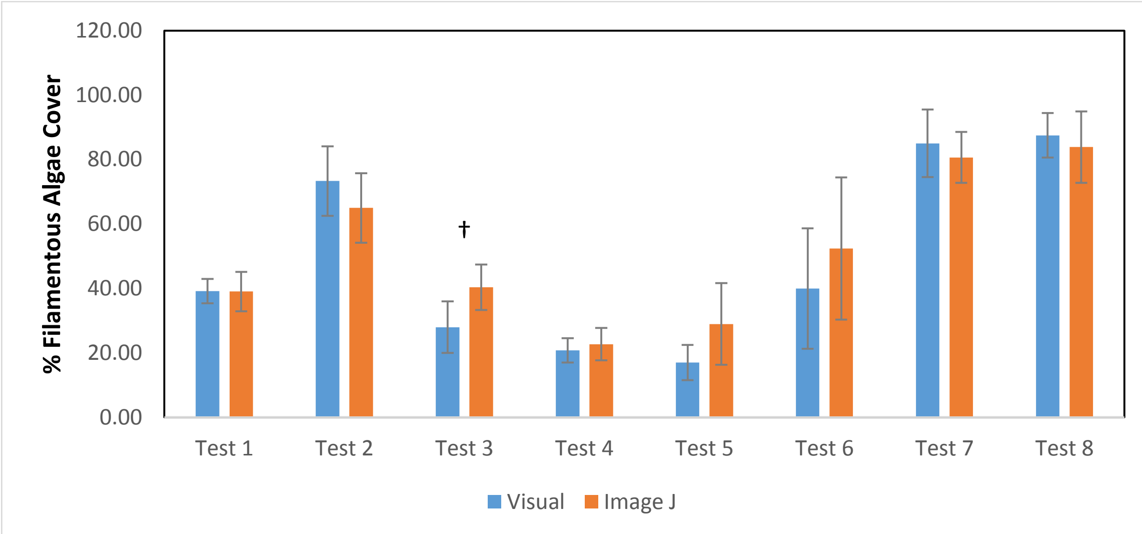

Figure 9 Bar graph of average percent filamentous algae cover from visual (blue) and ImageJ (orange) estimations. † Statistical difference observed ($p = 0.02$).

The visual and ImageJ estimates for the eight test images of filamentous algae yield similar results (**Figure 9).** In seven of the eight trials, the observer's visual estimations were statistically similar to their corresponding ImageJ estimates. Test Image 3 was the only image where the visual and ImageJ estimates were somewhat different ($p = 0.02$). There was no predictable under- or over-estimation.

Visual estimates showing the most agreement between observers, as well as between the pairs of visual estimates and ImageJ estimates, were produced from images where the algal patches were clearly different from the bottom substrate. Images such as Test Image 1, Test Image 4, Test Image 7, and Test Image 8 all had algal colonies with simple structure, and limited encroachment by periphyton and/or submerged aquatic vegetation. The largest differences between visual and ImageJ estimates were from images complicated by long shadows, periphytic mats, and senesced algae. Test Images 5 and 6 were attempts to apply this image processing method to measure filamentous algae as it begins on periphyton mats. ImageJ image processing is not recommended in cases where filamentous algae are overlapping periphyton mats.

The Intra-class Correlation Coefficients show that both the visual and ImageJ estimates yielded high observer agreement (**Table 4**), although greater agreement was found between observers for the visual estimates (0.897) than for the ImageJ estimates (0.793). The greater observer agreement for visual

Table 4 Interclass correlation coefficients (ICC) of the six user's visual estimations and ImageJ outputs of the proportion of algal cover in the datasets (white); result of paired t-Test comparison of visual estimates and ImageJ calculations (grey).

	Visual (ICC) between users	ImageJ (ICC) between users	Paired t-Test
Raw Observations	0.897	0.793	NS ($p < 0.10$)

observations may be due to 47 of 48 (i.e., 6 x 8) visual estimates of algal cover unintentionally being evaluated in 5% increments by the observers (e.g., 25%, 30%, 35%...). The unintentional rounding of the visual estimates likely created a higher level of observer agreement. This finding suggests that many observers are more comfortable estimating algal cover using a bin, represented by the rounded number, rather than a specific value. This rounding effect is not found with ImageJ because the application is manipulated along continuous gradients of color, brightness and hue until the user feels he/she has delineated the algae cover. The ICC for the ImageJ estimates was also extremely high (0.793), suggesting that users were delineating similar amounts of algae cover. A paired t-test found no discernable difference ($p < 0.10$, n = 48) in the visual estimates of algae cover and the ImageJ calculations of algae cover. Although the ImageJ protocol showed greater variance in predicting algal cover, there was general agreement in predicting algae cover between methods.

D. Potential Applications of Algae Cover Image Analysis

This analysis explored a digital image-based approach for verifying visual estimates, and is able to make several observations about the ImageJ application as a tool for estimating algal cover. ImageJ estimates were more variable than their corresponding visual estimates. This is likely due, in part, to users unintentionally rounding their estimates when making visual estimations. There is also user bias at the image processing stage when the "hue" and "brightness" scales are established. ImageJ is only able to distinguish between algae and other aquatic vegetation when there are differences in hue. A user may sacrifice or enhance what he/she perceives as filamentous algae when creating a "best fit" delineation of the algal cover with the ImageJ application. Due to these factors, bias may be introduced by what the user identifies as algae. For example, a user who visually estimates a high percent of algal cover in an image will often tend to delineate a large algal area in his/her ImageJ manipulations of the same image. A conservative user's low visual estimate will often translate to a smaller area of algae with the ImageJ application. This can result in a large amount of variation in the algal cover estimates between different users, but close agreement between the individual visual estimates and their corresponding ImageJ estimates.

Findings from these comparisons between the visual and ImageJ estimates of percent algal cover suggest some possible modifications to the field protocols:

- The agreement observed between the paired visual and ImageJ estimates suggests that ImageJ could be used as a training tool for field biologists new to quantifying algal cover.
- Established bins, in percent increments, for field observers to use in estimating percent algal cover, may enhance agreement between observer estimates.
- Due to the limited field of view of most underwater images taken in shallow water, multiple images could be used to create a mosaic to represent algal cover over a larger area.
- Future studies need to investigate image capture in large river systems, at greater depth, to determine the limits of visibility and resolution as depth increases.

One methodology for which ImageJ may hold high potential is hybridization with the viewing bucket procedure from EPA's Periphyton Rapid Bioassessment protocols. The viewing bucket procedure calls for a viewing bucket with the visible, submerged area sectioned into a grid. If a digital image is taken at the same time that algal estimates are made in the viewing bucket, post-processing or validation of the field measures could be performed using ImageJ protocols.

It is important to remember that the ImageJ application is still in its developmental stages as an algae percent cover analysis tool. The ImageJ protocol holds potential as an assessment tool, as an accessory protocol to existing methodologies, and as a validation tool for field data collected at wadeable sites, and may be useful as a training tool.

10. Summary of Findings

The first objective of this pilot study was to develop quantitative, repeatable, and scientifically valid methods for measuring filamentous algal growth in the Shenandoah River and its tributaries that can be applied in Virginia's other non-tidal (free-flowing) waters. It was apparent from the start that more than one method for estimating filamentous algae growth would be required in the Shenandoah River basin. While many of the basin's streams and small rivers are shallow and less than 100 m across, the larger mainstems are non-wadeable and can be very wide. The river mainstems also make numerous bends and meanders, and are not visible from roads much of the time.

Several sampling design aspects need to be considered before filamentous algae growth can be effectively located and measured in the river basin. A general reconnaissance of filamentous algae growth in the basin can identify affected reaches, where algal growth regularly occur, and can help focus efforts to detect and track algal blooms over time. The method(s) employed to measure filamentous algae will depend on the water's width and depth at a given sampling location. Field observers should receive training in aquatic plant identification, in order to correctly distinguish filamentous green algae from submersed aquatic vegetation (SAV) and other aquatic vegetation found in streams and rivers. Many of the underwater plant types appear similar from a distance or to the untrained eye. Analysis of water quality data collected at routine monitoring stations can be useful in identifying potential drivers and sources of algal blooms in a given river reach. A sampling season of May through September should be adequate for detecting most filamentous algae blooms.

The study found a previously developed, lateral transect method to be very suitable for estimating percent algal cover at sites in Shenandoah streams and wadeable rivers. Developed by WVDEP, the method has an established Standard Operating Procedure and has been tested in river systems neighboring the Virginia Shenandoah River. The WVDEP lateral transect method can be implemented rapidly by a two-person team when blooms are detected.

The study developed an adaptation of the WVDEP lateral transect method for sites on non-wadeable rivers. Canoes or small boats are used to navigate the deeper waters. The method requires skill in handling the craft, and it may be difficult to implement in rivers with many rock ledges and large boulders. The method produces results directly comparable to the WVDEP lateral transect method.

The two methods above were incorporated into a third design for the purpose of evaluating a length, or longitude, of a river. Longitudinal surveys are useful in finding and measuring algal growth in reaches of small and medium sized rivers that are not otherwise accessible. Estimates of algal cover are made along lateral transects when filamentous algae are encountered during the longitudinal survey. The method is time intensive and not practical for routine use. It should be employed in areas of known or suspected filamentous algal blooms, during peak growth seasons, and is best used to determine if algae growth exceeds established screening thresholds.

A fourth method developed for this study employs a subsampling design. It serves the purpose of estimating the areal extent of filamentous algae cover in a segment, or river reach. Measurements of percent algal cover made with a random or grid sampling design are more appropriate than the incidental, lateral transect measurements. The number and location of subsampling points in a segment will depend on whether a random or grid design is chosen, on the statistical confidence required in the estimate of percent algal cover, and on the amount of staff effort deemed appropriate for the task. A field trial of the method was successful, with close agreement in the visual estimates made by three independent field observers. The method can be particularly useful in evaluating inaccessible reaches of a river, and can be done more rapidly than the adapted, lateral transect methods.

The WVDEP lateral transect method and its two adaptations can address the question: “does the maximum extent of an algal bloom across a stream or river exceed a screening threshold?” The longitudinal survey with subsampling design can address the question “what percent of an entire segment is covered by filamentous algae?”

The second objective of this study was to investigate the feasibility of using citizen monitors to collect algal data in a manner acceptable to VADEQ and EPA. This objective was suggested in part due to the number of citizen reports of nuisance plant growth, and the presence of two well-organized and long-standing citizen monitoring organizations in the basin. A one-day training session was held on May 13, 2014, and was attended by citizen monitors as well as biologists from USEPA and several state agencies. Monitoring equipment for ten field teams were assembled into kits and delivered to the citizen monitoring organizations. Citizen monitors were invited to either monitor their already established sites, or other sites where they would be comfortable applying the wadeable methods.

The anticipated participation in the pilot volunteer effort during the summer of 2014 was not realized due to several factors. Many of the training session attendees did not feel comfortable with the physical demands of wading across sometimes swift streams. The work requires teams of at least two people and most citizen monitors are accustomed to working on their own. Some individuals who expressed interest in the program were unable to make the training date. The citizen monitors required more support than ICPRB staff, involved in other projects, were able to give them. Citizen monitors willing to participate in the project dropped out over time while new, untrained volunteers came forward, lending inconsistency to the effort. Finally, a significant amount of time passed between the May training session and manifestation of the algae blooms in the Shenandoah in 2014. The blooms peaked in late August and September. Lessons were learned and citizen monitoring involvement could prove successful in the future, with additional training of the volunteers. Citizen monitors in the Shenandoah River basin have already demonstrated they can serve as “eyes on the river,” and if trained individuals have a reliable way of reporting occurrences of filamentous algae, they might provide some of the reconnaissance needed for in a filamentous algae monitoring program.

The possibility of verifying visual estimates of percent algal cover with digital photos of the same area was investigated. For this pilot study, the ImageJ image processing software was applied to photos of different levels of algal cover in the field and compared to visual estimates made from the same photos. Capturing high quality images of algal cover in the stream or river proved critical to obtaining useable digital images for estimate verification. The imaging processing technique shows promise as a means for increasing consistency between the visual estimates of different observers. It holds potential as an accessory protocol, as a validation tool for field data collected at wadeable sites, and may be useful as a training tool.

BIBLIOGRAPHY

Angradi, T.R. (editor). 2006. Environmental Monitoring and Assessment Program: Great River Ecosystems, Field Operations Manual. EPA/620/R-06/002. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/emap/greatriver/fom.html

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Bartko, J.J. 1966. The Intraclass Correlation Coefficient as a Measure of Reliability. Psychological Reports, 19, p 3-11.

Biggs, B.J.F., D.G. Goring, and V.I. Nikora. 1998. Subsidy and Stress Responses of Stream Periphyton to Gradients in Water Velocity as a Function of Community Growth Form. Journal of Phycology 34(4): 598–607.

Biggs, B.J.F. 2000. Eutrophication of Streams and Rivers: Dissolved Nutrient-Chlorophyll Relationships for Benthic Algae. Journal of the North American Benthological Society 19(1):17-__.

California's State Water Resources Control Board (SWRCB). 2008. Incorporating Bioassessment Using Freshwater Algae into California's Surface Water Ambient Monitoring Program (SWAMP) http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/563_PeriphytonBioassessmentSWAMP.pdf (Accessed January 28, 2015)

Chételat, J., F.R. Pick, A. Morin, and P.B. Hamilton. 1999. Periphyton Biomass and Community Composition In Rivers Of Different Nutrient Status. Canadian Journal of Fisheries and Aquatic Sciences 56:560-569.

Copp, G.H. (1997). Microhabitat Use of Fish Larvae and 0+ Juveniles in a Highly Regulated Section of the River Great Ouse. Regulated River: Resource and Management 13(313): 267–276.

Fetscher, A.E., L. Busse, and P.R. Ode. 2009. Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 002. (updated May 2010)

Flotemersch, J.E., J.B. Stribling, and M.J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. EPA 600-R-06-127. US Environmental Protection Agency, Cincinnati, Ohio.

Gamer, M., J. Lemon, and I. Fellows. 2012. Various Coefficients of Inter-rater Reliability and Agreement. R package version 0.84. http://CRAN.R-project.org/package=irr

Griggs, A. N., J. Cummins, and C. Buchanan. 2012. Filamentous Algae Monitoring Pilot Program: West Virginia Rivers of the Potomac River Basin. Report prepared by ICPRB for the West Virginia Department of Environmental Protection, Division of Water and Waste Management. ICPRB Report 12-07. Available online at: http://www.potomacriver.org/pubs/pubs.

Griggs, A. N., and J. Cummins. 2014. Filamentous Algae Monitoring Program: Potomac River Basin. Report prepared by ICPRB for the West Virginia Department of Environmental Protection, Division of Water and Waste Management. ICPRB Report 14-01. Available online at: http://www.potomacriver.org/pubs/pubs.

Hallgren, K.A. 2012. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. Tutorials in Quantitative Methods for Psychology 8(1):23–34.

Hilton, J., M. O'Hare, M.J. Bowes, and J.I. Jones. 2006. How Green is My River? A New Paradigm of Eutrophication in Rivers. Science of the Total Environment 365(1–3): 66-83.

Klapproth, J. & J.E. Johnson. 2000. Understanding the Science Behind Riparian Forest Buffers: Effects on Plant and Animal Communities. Virginia Cooperative Extension, Virginia Polytechnic Institute and State University, Blacksburg, VA. p 420-152.

Koutsoukos, P. 2000. Current Knowledge of Calcium Phosphate Chemistry and in Particular Solid Surface- Water Interface Interactions. Institute of Chemical Engineering and Chemical Process. University of Patras, Greece.

Lazorchakl, J.M., A. Herlihy, J. Green. 1998. USEPA EMAP: Section 14 Environmental Monitoring and Assessment Program-Surface Waters: Field operations and Methods for Measuring the Ecological Condition of Wadeable Streams. Available: http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/Sec14.PDF

Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo & S.E. Charlton. 1984. “River Epilithon: Toward a Structural-Functional Model” Oikos 42(1):10-22. Available: http://www.jstor.org/discover/10.2307/3544604?sid=21105671243453&uid=2134&uid=2129&uid=2&uid=70&uid=4

Middleton, L. September 2013. “VA, Riverkeeper differ on how to handle algae in Shenandoah.” Bay Journal. Print and online at www.bayjournal.com.

Moulton, S.R., J.G. Kennen, R.M. Goldstein, and J.A. Hambrook. 2002. Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02-150. Available for download at http://pubs.usgs.gov/of/2002/ofr-02-150/

Quinn, J.M., and B.W. Gilliland. 1989. The Manawatu River Cleanup-Has it worked? Transactions of the Institution of Professional Engineers: Civil Engineering Section 16(1): 22-26. Available online at http://search.informit.com.au/documentSummary;dn=376511745638334;res=IELENG> ISSN: 0111-9508.

Quinn, J.M., & C.W. Hickey. 1990. Magnitude of Effects of Substrate Particle Size, Recent Flooding and Catchment Development on Benthic Invertebrate Communities in 88 New Zealand Rivers. New Zealand Journal of Marine and Freshwater Research 24:411-427. http://dx.doi.org/10.1080/00288330.1990.9516433.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, and M.R. Meador. 1993. Methods for Collecting Algal Samples as Part of the National Water-Quality Assessment Program: USGS Open-File Report 93-409.

Raven, J. 1992. How Benthic Macroalgae Cope with Fresh-Water Resource Acquisition and Retention. Journal of Phycology 28:133-146.

Schindler, D.W. 1977. Evolution of Phosphorus Limitation in Lakes. Science 21 January 1977: 260-262.

Stevenson, R.J., M.L. Bothwell and R.L. Lowe (editors). 1996. Algal Ecology: Freshwater Benthic Ecosystem, Academic Press. 752 p.

Summers, J., 2008. Assessment of Filamentous Algae in the Greenbrier River and Other West Virginia Streams. Report prepared for West Virginia Department of Environmental Protection, Division of Water and Waste Management.

Vannote R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37(1):130-137.

Wehr, J.D. and R.G. Sheath (editors). 2002. Freshwater Algae of North America: Ecology and Classification (1st edition). Academic Press.

Welch, E.B., J.M. Jacoby, R.R. Horner, and M.R. Seeley. 1988. Nuisance Biomass Levels of Periphytic Algae in Streams. Hydrobiologia 157: 161-168.

Wetzel, RG. 2001. Limnology — Lake and river ecosystems (3rd edition). Academic Press.

Whitford, L.A. 1969. The Current Effect and Growth of Freshwater Algae. Transactions of American Microscopical Society 79:302-309.

Whitford, L.A., and G.J. Schumacher. 1968. Notes on the Ecology of Some Species of Fresh-Water Algae. Hydrobiologia 32(1-2):225–236.